Claim 5 has been amended to delete the recitation of "in said mutated DNA." The amendment merely corrects for antecedent basis and, as such, addresses an informality and does not add new matter.

Regarding the Sequence Listing

In accordance with 37 C.F.R. §§1.821(c) and (e), submitted herewith are paper and computer readable copies of the sequence listing. The paper and computer readable copies of the sequence listing are the same and do not add new matter. Submitted concurrently herewith is an executed statement under 37 C.F.R. §§1.821(f) and (g) that the paper and computer readable copies of the sequence listing are the same and do not add new matter. Accordingly, no new matter has been added.

Thus, as the amendments to the specification and claim 5, and the paper and computer readable copies of the sequence listing do not add new matter, entry thereof is respectfully requested. Applicants respectfully request reconsideration of the present application.

I. OBJECTION TO THE SPECIFICATION

The specification stands objected to due to an informality. In particular, there appears to be text missing from the top right hand corners of application pages 2 and 3, which occurred during photocopying of the parent application.

As set forth above, the specification has been amended to insert the missing text at the top right hand corners of pages 2 and 3. In view of the amendment to the specification, Applicants respectfully request that the objection be withdrawn.

II. REJECTIONS UNDER 35 U.S.C. §112

The rejection of claims 1 to 9 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is respectfully traversed. The grounds for rejection are due to the meaning of "accompanying 5' untranslated region," "a portion of intron 1" (claim 2), and antecedent basis for "said mutated DNA" (claim 5).

Claims 1 to 9 are clear and definite as written. As to the meaning of "accompanying 5' untranslated region," the specification discloses that the "rAAV vector of the invention also comprises 5' and 3' untranslated regions of DNA which flank the hF.IX DNA sequence" (page 14, lines 19-20). Thus, the skilled artisan would understand that a 5' untranslated region could be a sequence located 5' of genomic F.IX coding sequence. The specification also discloses that "other 5' and 3' untranslated regions may be used in place of those recited in the case of hF.IX" (page 15, lines 5-7). Thus, in view of the specification, the skilled artisan would understand that a 5' untranslated region may be a sequence different from that located 5' of genomic F.IX coding sequence. As to the recitation of a "promoter/regulatory sequence," the specification exemplifies the CMV promoter enhancer sequence located at the 5' end of the F.IX sequences (page 14, lines 22-24). Thus, in view of the specification, the skilled artisan would understand that a promoter/regulatory sequence is in addition to the 5' untranslated sequence (e.g., located 5' of a 5' untranslated region of genomic F.IX coding sequence). Accordingly, claim 1 is clear and definite.

As to the meaning of "a portion of intron 1" in claim 2, the specification discloses that this is a region which "enhances expression of F.IX by at least about 1.5 fold on a plasmid or viral vector template" (page 14, lines 13-17). Thus, in view of the specification, the skilled artisan would understand that the "portion of intron 1" is located on the plasmid or viral vector. Accordingly, claim 2 is clear and definite.

Claim 5 has been amended to delete the recitation of "in said mutated DNA." Thus, in view of the amendment the grounds for rejection of claim 5 are moot.

In sum, in view of the specification and the amendment, claims 1 to 9 would be understood by the skilled artisan. As such, claims 1 to 9 are clear and definite and Applicants therefore respectfully request that the rejection under 35 U.S.C. §112, second paragraph be withdrawn.

III. REJECTIONS UNDER 35 U.S.C. §§102 and 103(a)

The rejection of claims 1 to 3 and 6 to 8 under 35 U.S.C. §102(a) as allegedly anticipated by Wiener *et al.* (WO 96/15777) is respectfully traversed.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration (In re Spada, 15 USPQ 2d 1655 (Fed. Cir. 1990), In re Bond, 15 USPQ 2d 1566 (Fed. Cir., 1990).

Applicants submit that Wiener *et al.* (WO 96/15777) do not teach each and every element of the claimed invention. Nevertheless, in order to expedite prosecution, submitted herewith is a sworn declaration under 37 C.F.R. §1.131 executed by Dr. Katherine High, one of the co-inventors of the subject application (Exhibit 1). The declaration states that the recombinant AAV vector including DNA encoding Factor IX was first constructed prior to the May 30, 1996, publication date of Wiener *et al.* (Exhibit 1, paragraphs 5 and 6). Evidence of the conception and reduction to practice of the recombinant AAV vector including DNA encoding Factor IX is supplied in the form of a copy of a page a laboratory notebook (Exhibit 2), prior to October 3, 1995. This page shows a diagram of the recombinant AAV vector including DNA encoding Factor IX (pSSV-F.IX intron; symbols are as follows: CMV=CMV promoter; F.IX = the human factor IX gene with a portion of intron 1; SV40 = polyadenylation signal; and Nde I restriction sites), as well as transformed clones that contain the vector.

Thus, as the present invention was reduced to practice prior to the publication date of Wiener *et al.* (WO 96/15777), this cited reference is not available as prior art against claims 1 to 9. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §102(a) be withdrawn.

The rejection of claims 1 to 3 and 6 to 8 under 35 U.S.C. §102(e) as allegedly anticipated by Wilson *et al.* (U.S. Patent No. 5,866,552) is respectfully traversed.

Applicants submit that Wilson *et al.* (U.S. Patent No. 5,866,552) do not teach each and every element of the claimed invention. Nevertheless, in order to expedite prosecution, a sworn declaration under 37 C.F.R. §1.131 executed by Dr. Katherine High, one of the co-inventors of the subject application is submitted herewith (Exhibit 1). As discussed above, the declaration and accompanying Exhibit 2 indicate that the recombinant AAV vector including DNA encoding Factor IX was constructed prior to October 3, 1995, which is before the September 6, 1996, filing date of U.S. Patent No. 5,866,552. Thus, as the present invention was reduced to practice prior to the filing date of Wilson *et al.* (U.S. Patent No. 5,866,552), this cited patent is not

available as prior art against claims 1 to 9. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §102(a) be withdrawn.

The rejection of claims 1 to 3 and 6 to 9 under 35 U.S.C. §103(a) as allegedly unpatentable over Wiener *et al.* (WO 96/15777) in view of Crabtree *et al.* (U.S. Patent No. 5,834,266) is respectfully traversed.

Applicants submit that neither Wiener *et al.* (WO 96/15777) nor Crabtree *et al.* (U.S. Patent No. 5,834,266) alone, or in combination, teach or suggest the claimed invention. Nevertheless, in order to expedite prosecution, the sworn declaration under 37 C.F.R. §1.131 (Exhibit 1) and accompanying Exhibit 2 indicate that the recombinant AAV vector including DNA encoding Factor IX was constructed prior to October 3, 1995, which is before the May 30, 1996, publication date of Wiener *et al.* Thus, Wiener *et al.* (WO 96/15777) is not available as prior art against claims 1 to 9. Crabtree *et al.* (U.S. Patent No. 5,834,266) do not teach or suggest claims 1 to 9. For example, as acknowledged by the Examiner, Crabtree *et al.* fail to teach or suggest Factor IX. Thus, claims 1 to 9 would not have been obvious in view of Crabtree *et al.* (U.S. Patent No. 5,834,266). Accordingly, Applicants respectfully request that the rejection of claims 1 to 3 and 6 to 9 under 35 U.S.C. §103(a) over Wiener *et al.* (WO 96/15777) in view of Crabtree *et al.* (U.S. Patent No. 5,834,266) be withdrawn.

The rejection of claims 1 to 3 and 6 to 9 under 35 U.S.C. §103(a) as allegedly unpatentable over Skulimowski *et al.* (Methods in Molecular Genetics 7:3 (1995)) in view of Kurachi *et al.* (J. Biol. Chem. 270:5276 (1995)), Webster *et al.* (U.S. Patent No. 5,834,306), Thiell *et al.* (U.S. Patent No. 5,817,784), Kaufman (Methods Enzymol. 185:487 (1990)) and Roman *et al.* (Somat. Cell Mol. Genet. 18:247 (1992)) under 35 U.S.C. §103(a) is respectfully traversed.

Applicants submit neither Skulimowski *et al.* (Methods in Molecular Genetics 7:3 (1995)), nor Kurachi *et al.* (J. Biol. Chem. 270:5276 (1995)), Webster *et al.* (U.S. Patent No. 5,834,306), Thiell *et al.* (U.S. Patent No. 5,817,784), Kaufman (Methods Enzymol. 185:487 (1990)) and Roman *et al.* (Somat. Cell Mol. Genet. 18:247 (1992)) alone, or in any combination, teach or suggest the claimed invention. Nevertheless, in order to expedite prosecution, the sworn

declaration under 37 C.F.R. §1.131 (Exhibit 1) and accompanying Exhibit 2 indicate that the recombinant AAV vector including DNA encoding Factor IX was constructed prior to October 3, 1995, the publication date of Skulimowski *et al.* Evidence of the October 3, 1995, publication date of Skulimowski *et al.* is provided in the form of a copy of an email from a representative of Harcourt/Academic Press, the publisher of the reference in which the cited Skulimowski *et al.* article appeared (Exhibit 3). The cited Thiell *et al.* patent was filed on August 9, 1996, which is also after the construction of the recombinant AAV vector. Thus, neither Skulimowski *et al.* (Methods in Molecular Genetics 7:3 (1995)) nor Thiell *et al.* (U.S. Patent No. 5,817,784) are available as prior art against claims 1 to 9.

Kurachi *et al.* (J. Biol. Chem. 270:5276 (1995)), Webster *et al.* (U.S. Patent No. 5,834,306), Kaufman (Methods Enzymol. 185:487 (1990)) and Roman *et al.* (Somat. Cell Mol. Genet. 18:247 (1992)) *et al.* (U.S. Patent No. 5,834,266) do not teach or suggest claims 1 to 9. For example, as acknowledged by the Examiner, Kurachi *et al.* fail to teach or suggest AAV vectors; Webster *et al.* fail to teach or suggest Factor IX; Kaufman fails to teach or suggest AAV vectors or Factor IX; and Roman *et al.* fail to teach or suggest AAV vectors or a portion of intron 1. Thus, claims 1 to 9 would not have been obvious in view of Kurachi *et al.* (J. Biol. Chem. 270:5276 (1995)), Webster *et al.* (U.S. Patent No. 5,834,306), Kaufman (Methods Enzymol. 185:487 (1990)) and Roman *et al.* (Somat. Cell Mol. Genet. 18:247 (1992)) alone, or in any combination. Accordingly, Applicants respectfully request that the rejection of claims 1 to 3 and 6 to 9 under 35 U.S.C. §103(a) over Skulimowski *et al.* (Methods in Molecular Genetics 7:3 (1995)) in view of Kurachi *et al.* (J. Biol. Chem. 270:5276 (1995)), Webster *et al.* (U.S. Patent No. 5,834,306), Thiell *et al.* (U.S. Patent No. 5,817,784), Kaufman (Methods Enzymol. 185:487 (1990)) and Roman *et al.* (Somat. Cell Mol. Genet. 18:247 (1992)) be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 1 to 9 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4065.

Please charge any additional fees, or make any credits, to Deposit Account No. 03-3975.

Respectfully submitted,

Date: 6-5-01

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METHODS AND COMPOSITIONS FOR USE IN GENE THERAPY FOR TREATMENT OF HEMOPHILIA

CROSS REFERENCE TO RELATED APPLICATIONS

This is a divisional of U.S. application serial no. 09/038,910, filed 3/12/98, now U.S. Patent No. 6,093,392, which claims priority under 37 C.F.R. § 119(e) to Provisional Serial No. 60/040,711, filed March 14, 1997.

GOVERNMENT SUPPORT

This invention was supported in part by grants from the U.S. Government (NIH Grant Nos. R01 HL53688 and P50 HL54500) and the U.S. Government may therefore have certain rights in the invention.

FIELD OF THE INVENTION

The field of the invention is gene therapy for treatment of diseases involving a deficiency of proteins in the blood stream.

BACKGROUND OF THE INVENTION

The process of blood coagulation involves a series of proteins known as blood coagulation proteins which act in a cascade fashion to effect the formation of a blood clot. Hemophilia is a disease of humans and other mammals wherein a gene encoding a blood coagulation factor contains a mutation such that the encoded protein does not function normally in the cascade process. Specifically, the hereditary disease, hemophilia B, is characterized by a mutation in a gene encoding the blood coagulation protein, Factor IX (F.IX). F.IX is reviewed in High et al. (1995, "Factor IX" In: Molecular Basis of Thrombosis, and Hemostasis, High and Roberts, eds., Marcel Dekker, Inc.).

Adenoviral vectors are well known in gene therapy and have been used to effect expression of high levels of canine factor IX in immunodeficient mice or in immunocompetent mice when the virus is administered in conjunction with

immunosuppressive agents. When adenoviral vectors are administered to immunocompetent mice in the absence of immunosuppressive agents, these vectors induce a strong inflammatory and cytotoxic T lymphocyte (CTL) response (Dai et al., 1995, Proc. Natl. Acad. Sci. USA 92:1401-1405) which negates the beneficial effects of the therapy. In addition, there are reports which suggest that intramuscular injection of replication defective adenovirus provides long-term expression of a transgene, provided that the transgene encodes a self-protein (*i.e.,* a host protein), such that a strong host immune response is avoided (Tripathy et al., 1996, Nature Med. 2:545-550; Yang et al., 1996, Hum. Mol. Genet. 5:1703-1712). Thus, while there has been significant progress in the area of gene therapy in *in vivo* expression of a selected transgene following direct injection of an adenoviral vector into skeletal muscle, the use of adenoviral vectors may not be the optimal method for gene therapy in light of these immunological considerations.

Retroviral vectors have also been used experimentally as a model for treatment of hemophilia B. However, levels of expression of F.IX from these vectors are reported to be too low to be of therapeutic value (Kay et al., 1993, Science 262:117-119).

Plasmid DNA which has been injected into mouse muscle has been shown to direct expression of erythropoietin (Epo) (Tripathy et al., 1996, Proc. Natl. Acad. Sci. USA 93:10876-10880), but this method of gene therapy is apparently not sufficiently efficient for the expression of a gene product such as F.IX, which is needed at relatively high levels in the circulation (compared with Epo) to achieve a therapeutic effect.

Adeno-associated virus (AAV) is an alternative vehicle to adenovirus for delivery of genes to muscle. Recombinant AAV (rAAV) does not contain sequences encoding viral proteins and has the potential to integrate into the chromosomal DNA of the host cell (Carter, 1992, Curr. Opin. Biotech. 3:533-539; Skulimowski et al., 1995, Method Mol. Genet. 7:7-12). Production and purification procedures are now available which facilitate the generation of pure rAAV which is not

significantly contaminated by wild-type AAV or helper adenovirus (Skulimowski et al., 1995, supra; Fisher et al., 1996, J. Virol. 70:520-532; Samulski et al., 1989, J. Virol. 63:3822-3828). As noted herein, administration of adenovirus to mammals is accompanied by the aforementioned immunological problems.

While the efficiency of *in vivo* transduction with rAAV in the absence of helper virus is low for hepatocytes and airway epithelial cells (Fisher, 1996, supra), certain post-mitotic cells such as neurons (Kaplitt et al., 1994, Nature Genet. 8:148-154) and skeletal muscle fibers (Xiao et al., J. Virol. 70:8098-8108) can be effectively transduced with this vector. Stable expression of *lacZ* for up to 1.5 years has been reported (Xiao et al., supra). In contrast to adenoviral vectors, intramuscular injection with rAAV in immunocompetent animals does not result in a CTL response against transduced muscle fibers, nor are circulating antibodies against the intracellular *lacZ* gene product present.

The expression of the secreted protein, Epo, following intramuscular injection with rAAV is reported in Kessler et al. (1996, Proc. Natl. Acad. Sci. USA 93:14082-14087). However, the levels of protein expression reported were one to two orders of magnitude below that required for a therapeutic effect mediated by F.IX.

Current therapy for hemophilia involves the intravenous injection of a preparation of clotting factor concentrates whenever a bleed occurs. This treatment is cumbersome, inconvenient and very expensive. The average patient pays approximately $100,000 per year for the concentrate alone. Further, because the concentrate is only administered to the patient intermittently, patients remain at risk for life-threatening bleeds which are fatal if treatment is not timely administered.

There is a long felt and acute need for methods of delivering F.IX to mammals having hemophilia, in particular, to humans having hemophilia, such that a therapeutic effect is achieved. The present invention satisfies this need.